ANTIDIABETIC ACTIVITIES OF SELECTED SPECIES OF *SYZYGIUM*

A PhD WORK PLAN

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1. INTRODUCTION

Plants are used as medicines from the middle of Paleolithic age (Fabricant & Farnsworth, 2001). Of the 2, 50,000 higher plant species only 6% of plants are examined for biological activity and only 15% of them are evaluated phytochemically (Vlietinck & Berghe, 1991). Plants are the old and crucial source of medicine and 50% of the prescription drugs are derived from higher plants (Veeresham, 2012).

Plants provide 70% people needs for their primary healthcare purposes (WHO, 2002). Numerous medicinal plants have been reported to prevent and control many diseases. WHO listed 21,000 plants, which are used for medicinal purposes throughout the world. Among these 2500 species are in India. India is the largest producer of medicinal herbs and is called the botanical garden of the world (Swaroopa & Code, 2017). Natural products play an important role in drug development. With least side effects and low-cost make the herbal preparations play a major role in the most of the therapies.

Family Myrtaceae is native to tropical region with about 130genera and 4000 species (Souza & Lorenzi, 2005). They include evergreen, woody shrubs to tall trees, with entire, aromatic leaves containing oil glands known to contain essential oils (Wilson *et al.,* 2001). *Syzygium* (Myrtaceae) is one of the largest genus of Angiosperms with 1200 species (Mabberly D.J, 2008) and in India there are 40 species (Punekar, 2011).

Members of the genus *Syzygium* have been used for a broad range of medicinal purposes by traditional healers from a wide variety of ethnic and cultural groups. Various species of *Syzygium* are used for a wide variety of medicinal purposes in traditional Indian medicinal systems, particularly in the Ayurveda. In India, *Syzygium aromaticum* and *Syzygium cumini* are abundantly used as a component of multiple traditional therapies for the treatment of many diseases. Many species are used for their antibacterial, antifungal, antiprotozoal, antiviral, antidiarrheal, antidiabetic, analgesic, antimalarial, antioxidant, anti-inflammatory, and anticancer activities. (Banerjee *et al.,* 2006; Jeyasankar *et al.,* 2011).

Diabetes is a metabolic disease characterized with hyperglycaemia as a result of defects in insulin secretion or insulin action (Nelson & Cox, 2010). The earliest mention of Diabetes (Madhumeha) was made by Sushrutha in 400 BC. Celsus (30 BC-50 BC) recognised the disease, and Cappadocia gave the name “Diabetes” (George & Cahill, 2005). In the 3rd to 5th centuries many scholars in China, Japan and India wrote diabetes as a condition with polyuria in which the urine was sweet and sticky.

Diabetic condition occurs mainly due to defect in insulin secretion, insulin action or both. Insulin deficiency leads to chronic hyperglycaemia by disturbing carbohydrate, fat and protein metabolism. With the progression of disease, severe diabetic complications such as retinopathy, nephropathy, cardiovascular complications and microbial complications begin to occur.

The world prevalence of diabetes among adults (aged 20-79 years) was estimated to be 6.4%, affecting 285 million adults, in 2010, and will increase to 7.7% and 439 million adults by 2030

(Shaw *et al.*, 2010). There are 425 million people around the world who have been suffering from diabetes in 2017. The Chinese population has the highest number of diabetic patients amounting to 114.4 million. India has the second largest number of diabetic patients, which are 72.9 million individuals during 2015. There has been around 50% increase in the diabetic patients in India between 2005 and 2015 (Jagetia, 2018).

Diabetes is of two types depending on the aetiology and clinical presentation. The insulin dependent diabetes mellitus (IDDM, Type I) and non-insulin dependent diabetes mellitus (NIDDM, Type II) (Sicree *et al.*, 2006) The type I diabetes (IDDM) is an autoimmune disease produced due to the destruction of β-cells of islets of Langerhans by the body's T lymphocytes leading to local inflammation and suppression of insulin secretion and this needs insulin replacement therapy (McCrimmon & Sherwin, 2010). It is more common in children than adults and has genetic predisposition and its incidence is on the rise (You & Henneberg, 2016). Type II diabetes is characterized by development of peripheral insulin resistance and impaired secretions of insulin by pancreas. It is more prevalent than type I (Herman & Zimmet, 2012).

Diabetes mellitus is considered as one of the five leading causes of death in the world (Vats *et al.*, 2004). There is no satisfactory effective therapy in modern medicine to cure diabetes mellitus (Ghosh & Suryawanshi, 2001). Due to side effects associated with the use of insulin and oral hypoglycaemic agents there is an increasing demand for natural products with antidiabetic activity (Holman & Turener, 1991; Rao *et al.*, 2001).World Health Organization (WHO) has recommended the evaluation of traditional plant treatments for diabetes as they are effective, non-toxic, with less or no side effects (Srinivasan, 2005).

In general *Syzygium* spp. are rich in phenolic compounds, terpenoids, flavonoids, anthocyanins, ellagic acid, isoquercetin, kaemferol, myricetin, tannins, etc. Phytochemical studies on seeds of *Syzygium cumini* revealed the presence of biologically active components in ethyl acetate and methanol extracts (Kumar *et al.*, 2009).Many reports have shown oxidative stress to be a major player in the depletion of antioxidant defense system in pancreatic islets (Nazirogilu, *et al.*, 2005; Bajaj and Khan 2012) and constitute a major factor in diabetic complications (Kwon *et al.*, 2006). Hence the antioxidant-rich agents may be helpful in preventing oxidative stress induced injuries (Oboh *et al.*, 2102).

In recent years the use of *Syzygium* species has greatly expanded by its potentiality for providing considerable beneficial effects on glucose metabolism and storage, and thus in diabetes treatment (Ayyanar & Babu, 2012).

2. LITERATURE SURVEY

The genus *Syzygium* is used for a wide variety of medicinal purposes in traditional Indian medicinal systems. Of the 40 species in India (Punekar, 2011), 26 species are endemic to the Western Ghats (Shareef *et al.,* 2010). Many species of *Syzygium* are well documented for their medicinal purposes.

Phytochemical analysis

Chemical components present in plants are categorized as primary and secondary metabolites based on their biological synthesis. Secondary metabolites present in the plants are principally responsible for treating various diseases. In general, *Syzygium* spp. are rich in phenolic compounds, terpenoids, flavonoids, anthocyanins, ellagic acid, isoquercetin, kaemferol, myricetin, tannins, etc.

Phytochemical studies on seeds of *Syzygium cumini* revealed the presence of biologically active components in ethyl acetate and methanol extracts (Kumar *et al.,* 2009).

Phytochemical extraction of *Syzygium polyanthum* leaf and fruit exhibited the presence of tannins, alkaloids, steroids, terpenoids and flavonoids while saponine was present in the ripened fruit extract and the extracts were having the potentiality towards antimicrobial and antioxidant activities (Kusuma *et al.,* 2011). Konczak *et al*. (2010) reported extremely high ascorbic acid levels in Syzygium *australe* and *Syzygium leuhmanii* (0.72 and 1.77μmol/g) respectively.

Syzygium aromaticum flower bud oil showed the presence of phytochemicals like monoterpenes, sesquiterpenes, phenols and hydrocarbon compounds where eugenol is one of the major phenylpropanoides (aromatic hydrocarbon) which make up 70-85% of clove oil and shown antimicrobial, antioxidant and anticancerous properties (Mittal *et al.,* 2014).

A comparative study of *Syzygium cumini* seed extracts indicated that methanolic extract was contained most of the phytochemicals and other bioactive compounds. In the quantification of phenols and proanthocyanins same methanolic extract had given maximum result while comparing with the rest (Atale *et al.,* 2011).

Different parts of *Syzygium cumini* showed the presence of various flavonoids and alkaloids, these are most importantly used to treat diabetes. Different flavonoids, terpenoids and phenolics such as quercitin, myricetin, kaempferol, sitosterol, etc. were reported in the leaves and bark of *Syzygium cumini* (Sharma *et.al.* 2006). Phytochemical analysis of *Syzygium cumini* seeds showed the presence of alkaloids, proteins, tannins, cardiac glycosides, flavons, saponins and steroids which showed α- amylase inhibition activity (Ponnuswamy *et. al.,* 2010).

A preliminary qualitative analysis of methanolic extract of *Syzygium polyanthum* leaf indicated the presence of tannins, glycosides, flavonoids and alkaloids and exerted its antihyperglycemic effect on streptozotocin induced diabetic rats (Widyavati *et.al.,* 2015).

From *Syzygium guineense* leaf extract, ten triterpenes were characterized and most of them have shown significant bioactivity (Djoukeng *et.al.,* 2005). Luo *et.al.,* (1999) have reported that terpenoids decrease the blood sugar levels in diabetic rats .

Syzygium jambos showed the greater concentration of phenolics. Administration of *Syzygium jambos* extract showed better blood glucose modulation over time. Bark extracts of the same species showed high amount of tannins and better *in vivo* efficacy in lowering blood glucose level (Gavillán-Suárez *et al.,* 2015).

From *Syzygium samarangense* sixteen flavonoids were isolated and they were successfully evaluated for immunomodulatory effect (Luo *et.al*., 2004). Significant reduction in plasma glucose level by the administration of different flavonoids in diabetic rats and stimulation of β- cells to release more of insulin were determined (Magno *et al.,* 2005).

Chemical composition and bioactivity of essential oil of *Syzygium travancoricum* was studied by Radha *et al.,* (2002). Trans – β- Ocimene, Trans- β- caryophyllene (Jirovetsz *et al.,* 1999), α- humulene, α -farnesene and allo-aromadendrenelinalool, nerolidol, terpineol, farnesol and pinocarveol like sesquiterpenes and oxygenated terpenes were reported and also they studied the antibacterial and antifungal activities of the oil.

The leaves of *Syzygium cumini* were known to contain ß-sitosterol, betulinic acid, mycaminose, crategolic acid, n-hepatcosane, n-nonacosane, n-hentriacontane, noctacosanol, n-triacontanol, n-dotricontanol, quercetin, myricetin, myricitrin and flavonol glycosides, myricetin, 3-O-(4'-acetyl)-L rhamnopyranosides and acylated flavonol glycosides (Rastogi & Mehrotra, 1995). Shafi *et al.,* (2006) reported that the leaf essential oil of *Syzygium cumini* was rich in pinocarveol, a-terpeneol, myrtenol, eucarvone, muurolol, amyrtenal, cineole, geranyl acetone, a-cadinol and pinocarvone.

Essential oil compositions of six *Syzygium* species were reported by Vinodkumar (2015). The leaf essential oil yield (%v/w) was recorded higher for *Syzygium zeylanicum* (0.33%), followed by *Syzygium hemisphericum* (0.17%), *Syzygium arnottianum* (0.12%) and *Syzygium lanceolatum* (0.10%), while the yield was negligible for *Syzygium laetum* (0.01%) and *Syzygium caryophyllatum* (0.01%).

Chang *et al.,* (1999) have isolated a new natural product, 4-epifriedelin (1), and 12 known terpenoids from the leaves of *Syzygium formosanum*. The known compounds include caryophyllene oxide, friedelin, canophyllal, glutinol, R-terpineol, phytol, betulinic acid, uvaol, lupeol, betulin, ursolic acid, and oleanolic acid. Betulinic acid, a pentacyclic lupane-type triterpene derived from the bark of *Syzygium formosanum* was evaluated for its cancer preventive property (Nakagawa-Goto *et al.,* 2009).

The hydro-distilled essential oil obtained from the aerial parts of *Syzygium kanarense* was reported by Joshi *et al.,* (2017). Fifty-two constituents were evaluated, which represented 91.9% of the total oil. The major compounds reported were seychellene (7.3%), α-muurolol (5.4%), cis-cadinene ether (5.3%), β- vetivenene (5.1%), 10 epi-γ-eudesmol (4.8%), guaiol (4.5%), longiborneol (4.1%), δ-elemene (4.1%) and α-muurolene (4.1%). The oil was found to be rich in sesquiterpene hydrocarbon (49.5%) type constituents.

Antioxidant potential

Change in the balance between oxidants and antioxidants is called oxidative stress and it leads to biological damage (Sies, 1985). Free radicals are generated during normal metabolism and during stress. This oxidative stress will be increased during chronic diseases such as diabetes and also with aging (Jakus, 2000). In diabetic condition, various reducing sugars such as glucose-6-phosphate and fructose increases through glycolysis and the polyol pathway that leads to an increase in reactive oxygen species (ROS) production and tissue damage (Matsuoka *et al.,* 1997). Many studies have shown oxidation stress mainly by the generation of free radicals in hyperglycemic condition and that contributed in the development and progression of diabetes (Giugliano *et al.,* 1996, Ceriello, 2003).

In diabetics, free radical production increased due to glucose autoxidation and other minor factors like reduction in enzyme action, increased levels of pro-oxidants such as ferritin and homocysteine. Elevated levels of advanced glycation end products (AGEs) also plays a major role in free radical production (Penckofer *et al.,* 2002). Some studies have indicated that reactive oxygen species play an important role in diabetic nephropathy. In high glucose condition glomerular mesangial cells produces increased amount of hydrogen peroxide also activation of secondary mediators like protein kinases increases that leads to oxidation stress-induced renal injury (Anjaneyulu & Chopra, 2004).

Antioxidants have been reported to reduce the complications in diabetes by arresting free radical damage (Fenercioglu *et al.,* 2010; Yokozawa *et al.,* 2002). Both enzymatic and non-enzymatic strategies are involved in antioxidant defence mechanism. Vitamins like A, C and E, enzymes like glutathione peroxidase and glutathione reductase function as antioxidants. By increasing the dismutation of oxygen radicals and clearing organic peroxides generated in diabetic condition, antioxidant enzymes protect biological system from oxidative stress (Pari & Latha, 2005).

Superoxide dismutase is one of the antioxidant enzymes that protects the cell against destructive effects of free radicals and scavenges superoxide by catalysing its dismutation to hydrogen peroxide and oxygen (Fukai & Fukai, 2011).Reduction in the imbalance between generation of free radicals and the antioxidant enzyme activities decreases the oxidative stress (Ajiboye *et al.,* 2018).

A significant relationship between antioxidant capacity and total phenolic content was reported by Reynertson *et al.,* (2002) in Myrtaceae fruits. A comparative study of *Syzygium aquem* dried and fresh fruit samples on its antioxidant activity had proven that fresh samples will give a higher antioxidant activity than the dried samples (Oman *et al.,* 2009). *In vitro* effect of *Syzygium cumini* was assessed for its radical scavenging activity and it was proven that the extracts had a preventive and protective effect in oxidation damage progression associated with diabetes (Raffaelli *et al.,* 2015). Oxygen free-radical can initiate peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of enzymes and alteration in the structure and function of collagen basement and other membranes, and play a role in the long term complication of diabetes (Sabu and Kuttan, 2002; Atawodi, 2005). Oboh *et al.,* (2015) found

the essential oil in *Syzygium aromaticum* to be rich in α-pinene, β-pinene and 1-8, Lineole which probably alleviates type 2 diabetes- related oxidative stress.

2, 2-Diphenyl-1- picrylhydrazyl (DPPH) is a widely used free radical used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity (Kirtikar & Basu, 2005). DPPH, a stable free radical, on reduction gives purple colour (Warrier *et al.,* 1994). Silver nanoparticles biosynthesised using *Syzygium cumini* seed extract were evaluated for their antioxidant property and those nanoparticles were found to have higher antioxidant capacity compared to the seed extract (Banerjee & Narendhirakannan, 2011). Adefegha & Oboh, (2012) reported that high DPPH radical scavenging ability of *Syzygium aromaticum* extracts was due to the presence of high total phenolic contents. Bajpai *et al.,* (2005) have observed that the hydromethanolic extract of *Syzygium jambolanum* seed was effective in scavenging (90.6%) free radicals as evaluated in the autoxidation of b-carotene and linoleic acid assay. Animal studies have also shown that administering *Syzygium jambolanum* seed decreased the levels of lipid peroxides in liver, kidneys, and serum of diabetic animals (Ravi *et al.,* 2004). A comparative study of *Syzygium densiflorum* on its antioxidant activity (DPPH assay) using the ethanol extract showed a maximum inhibition compared to the ethyl acetate and n-hexane. The ethyl acetate and ethanol extract had shown maximum inhibition at 0.2 mg/mL concentration than the L-ascorbic acid standard (Krishnasamy & Muthusamy, 2015). Islam *et al.,* (2013) determined the antioxidant activities of *Syzygium fruticosum* fractioned plant extract, where ethyl acetate fraction had shown the maximum antioxidant activities.

Assessment of oxidative stress by determining the antioxidant enzyme levels in *Syzygium cumini* was reported by Ajiboye *et al.,* (2018) where superoxide dismutase, catalase and glutathione peroxidase enzyme activities are compared with free and bound phenolic extracts using the standard drug metformin. Higher antioxidant enzyme activities were recorded in the plant extract than the standard drug.

Radical scavenging activity of *Syzygium lanceolatum* leaf essential oil was determined spectrophotometrically by Muthumperumal *et al.,* (2016), where the plant exhibited strong DPPH free-radical scavenging activity i.e. 69.97 % and Gallic acid was used as a standard synthetic antioxidant.

The total phenolic & flavonoid contents of *methanol and water extracts of* the leaves of *Syzygium malaccense* were studied by Savitha *et al.,* (2011). A comparatively high amount of phenols and flavonoids were reported in methanol extract than the water extract. The same extracts were subjected to their antioxidant studies and the plant shown high antioxidant activities and the higher inhibitory effect was reported in the methanolic leaf extracts.

The methanolic & aqueous extract of fruits of *Syzygium caryophyllatum* and *Syzygium zeylanicum* were studied for their antioxidant activities. Both the plants have shown significant antioxidant potentiality where ascorbic acid was used as standard (Shilpa & Krishnakumar, 2015).

Leaf and bark methanolic extracts of *Syzygium mundagam* were evaluated for their antioxidant and antidiabetic activities in streptozotocin induced male diabetic rats. Both the plant parts

have shown antioxidant and antihyperglycemic activities with high phenol, tannin and flavonoid content (Chandran *et al.,* 2017).

Cytoprotective and antioxidant effects of *Syzygium gratum was reported by Senggunprai et al., (2010).* The aqueous and ethanolic extracts of leaf have shown antioxidant and intracellular oxygen radical scavenging activity. The activity of heme oxygenase - cytoprotective enzyme, was significantly high in mice treated with *Syzygium gratum leaf extract and which is* beneficial against oxidative stress and other related disorders.

Antidiabetic activity

Diabetes mellitus is a metabolic disorder of the endocrine system. The occurrence of disease is worldwide. People suffering from diabetes are not able to produce or properly use insulin in the body. So they have a high level of blood glucose. In Indian traditional medicine system, diabetes is treated by using antidiabetic medicinal plants, but in general, treatment of diabetes is by oral hypoglycaemic agents and insulin (Chattopadhyaya *et al.,* 1993).

Many authors have evaluated the potentiality of *Syzygium* plant extracts and purified compounds in treating diabetes and diabetes-related diseases. It has been reported that the fruits of *Syzygium* sp. have been used to lower the blood glucose level during hyperglycaemia three decades prior to the discovery of insulin (Helmstadter 2008). Earlier, decoctions of *Syzygium cumini* leaves were directly used as hypoglycaemic agents (Brahmachari & Augusti, 1962), later researchers began to study the extracts and their effect on animal model system.

Shahreen *et al.,* (2002) reported that ethanolic extracts of *Syzygium cumini* induce pancreatic insulin secretion in diabetic rats. In rats induced with diabetes using streptozotocin, 100 mg/kg doses of ethanolic extracts of Syzygium *cumini* kernel restored normal blood glucose and liver glycogen levels compared to control animals and of diabetic animals treated with the hypoglycemic standard drug glibenclamide (Ravi *et al.,* 2004). Sharma *et al.,* (2008) reported that flavonoid-rich ethanolic extracts of *Syzygium cumini* seeds showed hyperinsulinemia and extracts from the plant bark also contain the active ingredients.

Administration of *Syzygium cumini* extracts 30 minutes before oral glucose loading significantly suppressed ($p<0.001$) the rise in postprandial blood glucose levels in treated rats compared to control rats. Oral treatment of streptozotocin-induced diabetic with ethanol and aqueous extracts of *Syzygium cumini* for 3 weeks resulted in significant reductions in fasting blood glucose levels compared with diabetic controls (Tripathi & Kohli, 2014).

The antidiabetic potential of *Syzygium cumini* seeds was investigated on streptozotocin - induced diabetic rats. The oral administration of the extract showed a decrease in glucose level, total cholesterol, triglycerides, and LDL-cholesterol and an increase in insulin level and HDL-cholesterol level (Sharafeldin & Rizvi, 2015).Type II diabetic individuals were administered with the standardized *Syzygium cumini* seed powder which exhibited a reduction in fasting blood sugar, insulin resistance and elevation in HDL cholesterol at the end of 3rd month (Sahana *et al.,* 2010)

Bansode *et al.,* (2016) have studied the *in vitro* α-amylase inhibitory activity assay of *Syzygium cumini* which showed that ethanol extract has the highest value of percent inhibition (73.33%) compared to standard drug Acarbose (65.99%).

From *Syzygium cumini* seeds 6 compounds were isolated (d- maslinic acid (1), 5-(hydroxymethyl) furfural (2), gallic acid (3), valoneic acid dilactone (4), rubuphenol (5), and ellagic acid (6)) and evaluated for antidiabetic properties using aldose reductase and protein-tyrosine phosphatase 1B inhibition assays. The isolated constituents showed a promising *in vitro* antidiabetic activity (Sawant *et al.,* 2015).

Mycaminose (50 mg/kg) - a compound isolated from *Syzygium cumini* seeds and ethyl acetate & methanol fraction at doses of 200 and 400 mg/kg body weight (b.w) showed a significant decrease in blood glucose level when evaluated for the anti-diabetic activity in streptozotocin -induced diabetes in rats (Kumar *et al*.s, 2013).*Syzygium cumini* seed extracts possesses α-amylase inhibitory activity which have been found to be linked with various flavonoid constituents.

The flavonoids isolated from the leaves of *Syzygium samarangense* had shown antihyperglycemic activity in alloxan induced diabetic rats (Baliga *et al.,* 2013).

Phytochemical examination of *Syzygium jambolanum* have confirmed the presence of flavonoids and they were shown to regenerate the damaged pancreatic β- cells in streptozotocien induced diabetic rats (Baliga *et al.,* 2013).

Antidiabetic activity of *Syzygium calophyllifolium* in Streptozotocin-Nicotinamide induced diabetic rats were studied by Chandran *et al.,* (2016). The rats were treated with 100 and 200 mg/kg of the *Syzygium calophyllifolium* bark methanol extract and compared with the diabetic, normal and standard glibenclamide groups and 200 mg/kg dose were much effective to reduce blood glucose level after 28 days of observation. The same study revealed that higher dose of extract could normalize the triglycerides, HDL, cholesterol and VLDL constituents in blood serum to the levels almost similar to that of normal rats.

The methanol extract of *Syzygium polyanthum* leaf proved to be the most effective anti-hyperglycaemic preparation. Blood glucose level in water extract and methanol extract loaded group of Wistar rats have shown significant reduction at 30 minutes after glucose loading, where metformin used as a standard drug (Widyavati *et al.,* 2015). Widharna *et al.,* (2015) reported that the oral administration of the extract mixture of *Andrographis paniculata* and *Syzygium polyanthum* resulted to a significant decrease on the levels of blood glucose in alloxan-induced diabetic Wistar rats compared to groups treated with single extracts. The histopathological studies carried out indicated that the extract mixture preserved pancreatic islet cells in alloxan-induced diabetic rats. A comparative antioxidant and antidiabetic studies of *Syzygium polyanthum and Momordica charantia* were carried out. A fresh fruit juice extract of *Syzygium polyanthum* demonstrated better free radical scavenging and antidiabetic activity than *Momordica charantia*. It was observed that the % inhibition of DPPH by fresh juice of *Syzygium polyanthum* was 64.93 similar to standard chemical quercetin 69.21 (Sandikapura *et al.,* 2018)

Emulsions of *Syzygium aromaticum* essential oil were evaluated for its antidiabetic potential and exhibited a maximum antidiabetic activity with 95.30% of α-amylase inhibition (Tahir *et al.,* 2016). Studies conducted by Khathi *et al.,* (2013) have shown that *Syzygium aromaticum*-derived oleanolic acid (OA) and maslinic acid (MA) reduce blood glucose concentrations in streptozotocin-induced diabetic rats. The administration of OA and MA reduced food intake, blood glucose levels and plasma ghrelin levels in streptozotocin induced diabetic rats and the control of food intake through the reduction of ghrelin expression (Luvuno *et al.,*2016) by plant derived OA and MA constituted a path of glycaemic control in diabetes mellitus.

Krishnasamy *et al.,* (2016) carried out a research with the ethanol fruit extract of *Syzygium densiflorum* to evaluate the antidiabetic properties in strptozotocin and nicotinamide -induced rats. Blood glucose, initial and final body weight, haemoglobin, total protein, HDL cholesterol and triglycerides were analysed. Decrease in the amount of blood glucose, cholesterol and triglycerides were observed. After 28 days of treatment there was an increase in the amount of high density lipoprotein and haemoglobin content. Due to reduced insulin sensitivity and impaired glucose homeostasis (Hermansen & Mortensen 2007) diabetics show increased body weight. The administration of 800mg/kg b.w of plant extracts had shown a considerable decrease in the body weight of rats treated with strptozotocin and nicotinamide.

The blood glucose lowering effect of *Syzygium guineense* leaf extract was observed in the fasting glucose level test (Lorenzati *et al.*, 2010). The potentiality of *Syzygium guineense* leaf extract (methanol) was evaluated by Ezenyi *et al.,* (2016). A dose of 250 mg/kg of extract significantly reduce the blood glucose level compared with standard drug metformin and the group also showed increase in hepatic glycogen content by 33.9% relative to the diabetic control. Serum biochemical analysis showed that the extract improved the kidney and liver functions by reduction in serum albumin, creatinine, liver enzymes, total and direct bilirubin.

Administration of bark and leaf extracts of *Syzygium mundagam* (200 mg/kg b.w.) had shown a significant reduction in blood glucose levels by 59.70% in strptozotocin induced diabetic rats (Chandran *et al.,* 2017).

Attanayake *et al.,*(2013) have reported that the effect of different doses of aqueous stem bark extracts of *Syzygium caryophyllatum* possess potent acute antihyperglycemic activity in alloxan induced diabetic rats and the optimum effective dose on glucose tolerance was found to be 1.00 g/kgb.w. in diabetic rats.

The aqueous and alcoholic extracts of dried bark of *Syzygium malaccense* were compared with glibenclamide for their influence on fasting blood sugar, lipid profile and liver glycogen in both normoglycemic and to streptozotocin induced (50mg/kg ip) hyperglycemic rats by Bairy *et al.,* (2005). A Significant lowering of total cholesterol, blood sugar and rise in HDL cholesterol and liver glycogen were reported in both aqueous and alcoholic extracts of bark.

Antidiabetic activity of *Syzygium calophyllifolium* bark methanol extract in Streptozotocin-Nicotinamide induced Type-2 diabetic rats was evaluated by Chandran *et al.,* (2016). The blood glucose level, biochemical and histopathological parameters were analysed. The best results

were reported for bark methanolic extract of 200mg/kg body weight and the same dose will protect the cellular architecture of liver kidney and pancreas were also revealed.

Pollens of *Syzygium cumini* are screened for their phytochemical constituents. The pollen part shown the presence of alkaloids, steroids, flavinoids glycosides, tannins and saponins which is reported to have the antidiabetic property (Ghoshal & Saoji, 2013).

Effect of leaf aqueous extract of *Syzygium cordatum* on blood glucose concentrations (mmol/L) of normoglycaemic rats was evaluated by Deliwe & Amabeoku, (2013). A significant reduction (23.37%) in the blood glucose of the fasted diabetic rats was reported in the 4^{th} h of observation after the treatment (12.50- 25 mg, p.o.).

Syzygium aqeuem leaf extracts possess α-amylase and α-glucosidase inhibitory properties, which has been found to be linked to multiple flavonoid constituents contained in the ethanolic fractions (Manaharan *et al.,* 2012) and these compounds prevent glucose uptake and enhance adipogenesis (Manaharan *et al.,* 2013)

From the stem bark of *Syzygium alternifolium* Trimurtulu *et al.,* (2015) have isolated two terpenoids friedelin and 3β-friedelinol. They reported that the isolated compounds while screening for *in vitro* enzyme inhibition assays against α-glucosidase and α-amylase and the extract displayed decreased plasma glucose level significantly showing antidiabetic activity.

Vescalagin, was identified in the young fruits of *Syzygium samarangense* and found to possess hypoglycemic effects and demonstrated to be effective in ameliorating glucose homeostasis in rats fed with high-fructose diets (Huang *et al.,* 2016; Shen & Chang, 2013).

Blood glucose in diabetic rats was significantly reduced following the administration of *Syzygium cordatum* leaf extract and oral administration of the extract notably increased hepatic glycogen content by comparison with untreated streptozotocin-induced diabetic rats (Musabayane *et al.,* 2005).

There are many digestive enzymes in humans and among them the most important one is pancreatic alpha-amylase that catalyses hydrolysis of the alpha-1, 4 glycosidic linkages of the starch, amylopectin, amylose, glycogen, and numerous maltodextrins and is responsible for starch digestion. The other important enzyme is alpha-glucosidase or maltase which catalyzes the final step of the digestive process of carbohydrates mainly starch by acting upon 1,4-alpha bonds and producing glucose as the final product (Tundis, 2010). If there will be excess conversion of starch to sugars, it will increase the sugar level in blood, then the role of insulin will come into action by ordering cells to metabolize the excess sugar moieties and store as energy sources i. e. glycogen. This cycle is endlessly happening in a healthy person. But in some cases, due to excess activity of amylase enzyme and insulin deficiency or resistance to insulin, level of blood glucose rises which might results in hyperglycaemia. To control hyperglycaemia several studies on inhibition of amylase and glucosidase enzyme activities is being studied. Acarbose-an inhibitor is of microbial origin and it was the first alpha-glucosidase inhibitor commercially available for diabetes treatment which inhibits the activities of alpha-amylase, sucrase and maltase (Agarwal & Gupta, 2016; Horii *et al.,* 1986).

Syzygium cumini seed kernel extracts were evaluated for the inhibition of α-glucosidase. *In vitro* studies using the mammalian α-glucosidase from rat intestine showed the extracts to be more effective in inhibiting maltase when compared to the acarbose control and the acetone extract was found to be a potent inhibitor of α-glucosidase (Shinde *et al.,* 2008).The interaction of free and bound phenolic extracts *Syzygium aromaticum* assesse for its α-amylase and α-glucosidase inhibitory activity where both the extracts showed significant values (Adefegha & Oboh, 2012). Ethanolic extracts of *Syzygium aqueum* leaf were investigated by Manaharan *et al.,* (2012) for its effectiveness in inhibiting the carbohydrate hydrolysing enzymes, a-glucosidase (EC50 = 11 lg/ml) and a-amylase (EC50 = 8 lg/ml) and showed high inhibitory activities on standard drug acarbose.

Kumar *et al.,* (2008) studied the acute toxicity of *Syzygium cumini* seed extract on albino Wistar rats by oral administration from lower to higher dose. The animals were kept fasting for overnight providing only water, after the extract administration, no mortality up to the dose of 2,000 mg/kg body weight was observed.

In streptozotocin induced diabetic rats due to the increased mobilization of free fatty acids from peripheral deposits, the lipid content of serum, liver and kidney will increase. Lipase is a hormone sensitive enzyme, in the presence of insulin its action is inhibited (Al-Shamaony *et al.,* 1994). By the administration of *Eugenia jambolana* seed kernel extract there was a significant decrease in the levels of total cholesterol, phospholipids, triglycerides and free fatty acids in the serum, kidney and liver were reported (Ravi *et al.,* 2005).

Glycosylated haemoglobin levels were monitored as a reliable index of glycaemic control in diabetes. Due to glycosylation of haemoglobin, the patients with diabetes mellitus will be having high amount of glycosylated haemoglobin (Babu *et al.,* 2005). Significant reduction in glycosylated haemoglobin was reported in the administration of methanol: water (3:1) fraction of *Syzygium alternifolium* seed extract in streptozotocin induced diabetic rats (Kasetti *et al.,* 2010).

Partial damage in the islets of Langerhans of pancreas was reported in alloxan- induced diabetic rats. After 30 days of treatment with 50- 70 mg/kg of b.w *Syzygium cumini* seed extract , all the β-cells which were partially damaged by alloxan injection had shown recovery and the recovery of damaged liver cells are also recorded (Singh & Gupta, 2007).

With this background, it is proposed to study some selected species of *Syzygium* for their antidiabetic activities.

3. PLAN OF WORK

OBJECTIVES OF THE STUDY

- To analyze qualitative and quantitative phytochemical properties of selected *Syzygium sp.* extracts.
- To assess *in vitro* antioxidant properties of the extracts.
- To evaluate antidiabetic activity of the extracts.

MATERIALS AND METHODS

- **Sample preparation**

Freshly collected samples will be cleaned, shade dried, powdered, sieved and stored in an air tight jar at room temperature.

- **Preparation of extract**

Dried powdered samples will be subjected to extraction using solvents such as Petroleum ether, chloroform, ethyl acetate, ethanol/methanol and water using soxhlation method and extract will be stored in a refrigerator at 0-4℃ until the use.

- **Preliminary phytochemical screening**

The extracts will be subjected to preliminary phytochemical screening to check the presence of alkaloids, flavonoids, phenols, saponins, glycosides, terpenes and steroids, following the methods given in Harborne, (1973)

Antioxidant potential of plant extract

This will be carried out by established standard methods

- ✓ DPPH assay (Mensor *et al*., 2001)
- ✓ SOD assay (Sajeeth *et al*., 2011)
- ✓ FRAP assay (Oyaizu, 1986)
- ✓ H_2O_2 scavenging assay (Ruch *et al*, 1989)
- ✓

1. **DPPH assay** (Mensor *et al*., 2001)

Procedure

The effect of extract on DPPH radical will be assayed using the method. A methanol solution of 0.5mL of DPPH (0.4mM) will be added to 1 mL of different concentrations of plant extract and allowed to react at room temperature for 30 minutes. Methanol will be serves as the blank and DPPH in methanol without the extracts serves as the positive control. After 30 min, the absorbance will be measured at 518 nm and converted into percentage radical scavenging activity as follows.

$$\text{Scavenging activity (\%)} = \frac{\text{A(518)control} - \text{A(518)sample}}{\text{A(518)control}} \times 100$$

Where, A (518) control is the absorbance of DPPH radical+ methanol and

A (518) sample is the absorbance of DPPH radical+ sample extract/ standard.

2. **SOD assay** (Sajeeth *et al*., 2011)

Procedure

1ml of NBT solution, 1ml of NADH solution, 0.1ml of plant extract (10mg in 0.1ml DMSO and 0.9ml PO_4 buffer) and 0.1ml of PMS solution will be added together and incubated at 25°C for 5 min. After 5 min the absorbance will be read at 560 nm.

3. **FRAP assay (**Oyaizu, 1986)

Procedure

The different concentrations of extract will be taken in 1.0 ml of distilled water & mixed with 2.5 ml of 0.2 M Phosphate buffer (pH 6.6), 2.5 ml of potassium ferricyanide (1%) and incubated at 50°C for 20 min. 2.5 ml of 10 % TCA will be added, centrifuged for 10 min at 1000 x g. To 2.5 ml of upper layer, 2.5 ml of distilled water and 0.5 ml of Ferric chloride (0.1%) will be added. Systems devoid of extract were chosen as control. The absorbance will be measured at 700 nm. The reducing power will be determined by comparing the absorbance of treatment with that of the control. Ascorbic acid (0.1%) used as standard.

4. **H_2O_2 scavenging activity** (Ruch *et al*, 1989)

Procedure

Solution of hydrogen peroxide (40mM) will be prepared in phosphate buffer pH 7.4 and its concentration will be determined by measuring the absorbance at 560nm using UV spectrophotometer. 0.1mg/ml of the extract will be added to hydrogen peroxide solution and absorbance measured at 560nm using UV spectrophotometer against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the extract and standard compound will be calculated using the given formula. Ascorbic acid solution will be taken as positive control.

Percentage scavenged [H_2O_2] = 1- Abs (standard)/Abs (control) x100

Where, Abs control is the absorbance of the control (without extract) at 560nm and

Abs sample is the absorbance in the presence of the extract at 560nm.

Antidiabetic studies

In-vitro assessment of antidiabetic property of plant extract will be done involving the following enzyme assays.

- ✓ α-Amylase inhibitory assay(Rao & Krishnamohan 2017)
- ✓ α-Glucosidase inhibitory assay (Khathi *et al.*,, 2013)

I. Enzyme assays

1. **α-Amylase inhibitory assay**(Rao & Krishnamohan, 2017)

Procedure

A series of concentrations of concentrations of plant extract (10, 40 and 80 µg/ml) will be prepared and allowed to react with α-amylase and 2 mM of phosphate buffer (pH- 6.9). After incubation for 20 min, to the reaction mixture 0.1 ml of 1% starch solution will be added. The same procedure will be followed for control samples without the enzyme. Finally, 0.5 ml of dinitrosalicylic acid reagent will be added to both control and test and kept in a boiling water bath for 5 min. Then, the absorbance will be measured at 540 nm using spectrophotometer and the percentage inhibition will be calculated using the formula,

$$\% \text{ Inhibition} = \frac{\text{Absorbance Control} - \text{Absorbance Test}}{\text{Absorbance Control}} \times 100$$

2. **α-Glucosidase inhibitory assay** (Khathi *et al.*,, 2013)

Procedure

1mL of 3mM *p*-nitrophenyl α-D-glucopyranoside (pNPG) in 0.2 M sodium phosphate buffer (pH 6.8) will be added as a substrate to the mixture of 50 µL of α-glucosidase (0.15 unit/mL), and 50 µL of sample to start the reaction. The reaction will be conducted at 37 °C for 15 min and stopped by the addition of 750 µL of 0.1 M Na_2CO_3. The α-glucosidase activity will be assessed by measuring the release of *p*-nitrophenol from pNPG at 410 nm. Glucosidase activity inhibition will be determined as percentage of inhibition, as follows.

$$\text{Inhibition } \% = 100\% - A_1/A_0$$

Where A_I is absorbance of sample solution, and A_0 is absorbance of control solution. Acarbose will be used as a positive control.

II. *In-vivo* assessment

- **Experimental animals**

Grown up adult male and female albino Wistar rats with body weight above 150g will be taken in separate groups. They will be housed at 12hours light dark cycles of standard environment with temperature $25 \pm 3^{\circ}C$. The rats will be fed with standard rat pellet diet and water *ad libitum*.

- **Acute toxicity test**

Acute oral toxicity study is performed as per OECD guidelines. Healthy groups of (n=3) albino Wistar rats starved overnight and orally fed with plant extract in increasing dose levels 50,100,250,500,1000 and 2000mg/kg of body weight. The rats will be observed continuously for 2 hours for changes in general behaviour and after 24 and 72h and up to 14 days for mortality. This is to be assess the plant extract dosage tolerance by the rats (Kumar *et al.*, 2013).

- **Oral glucose tolerance test**

Insulin mimicking activity of plant extract can be measured using Oral Glucose Tolerance Test (OGTT). The overnight fasted rats will be administrated with plant extract in low (300mg/kg body weight) and high (600mg/kg of body weight) doses. Citrate buffer will be used as vehicle control and Glibenclamide (5mg/kg body weight dissolved in 0.5ml of 0.9% saline) as positive control. After 30 minutes of standard and extract administration glucose at concentration of 2g/kg body weight fed into the entire group. Blood will be withdrawn from the retro-orbital-sinus prior and 30, 60, 120, 180, 240 minutes after the glucose administration and the glucose level will be measured using standard glucometer (Babu *et al.,* 2007)

- **Induction of diabetes**

Hyperglycaemia will be induced by a single intra peritoneal injection of freshly prepared 5mg/kg body weight of streptozotocin in 0.1M citrate buffer of pH 4.5 to overnight starved rats. After streptozotocin administration animals will be having free access to feed and water *ad libitum*. The diabetes in rats will be confirmed by the blood glucose level estimation of 72 hours post streptozotocin injection (Patel & Kori, 2018)

- **Experimental design**

The rats should be divided in to eleven groups (n=6) as below

Diabetes will be induced by a single intraperitonial injection of streptozotocin 5mg/kg

- **Group 1:** Normal non-diabetic control rats
- **Group 2:** Diabetic control rats – Streptozotocin induced (60 mg/kg b.w., p.o)
- **Group 3:** Diabetic rats+ Single dose of Glibenclamide (0.5mg/kg b.w., p.o.)
- **Group 4:** Diabetic rats+ Plant A extract 1 (low dose/kg kg b.w., p.o)
- **Group 5:** Diabetic rats+ Plant A extract1 (high dose/kg kg b.w., p.o)
- **Group 6:** Diabetic rats+ Plant A extract 2(low dose/kg kg b.w., p.o)
- **Group 7:** Diabetic rats+ Plant A extract 2 (high dose/kg kg b.w., p.o)
- **Group 8:** Diabetic rats+ Plant B extract 1 (low dose/kg kg b.w., p.o)
- **Group 9:** Diabetic rats+ Plant B extract1 (high dose/kg kg b.w., p.o)
- **Group 10:** Diabetic rats+ Plant B extract 2(low dose/kg kg b.w., p.o)
- **Group 11:** Diabetic rats+ Plant B extract 2 (high dose/kg kg b.w., p.o)

In all the experiments, body weight will be recorded at the commencement of the experiment (0 day) and weekly and on the day of autopsy.

III. **Biochemical studies**

Biochemical parameters involve Blood glucose estimation (Trinder, 1969), Creatinine estimation (Folin & Wu, 1919) and Estimation of glycosylated haemoglobin (Nayak & Pattabhiraman, 1981).

I. **Blood glucose estimation** (Trinder, 1969)

Blood samples will be collected by tail vein puncture at weekly intervals for a period of 28 days. Fasting blood glucose will be measured by glucose oxidase-peroxidase (GOD-POD) method in mg/dL using a digital glucometer.

I. **Creatinine estimation** (Folin & Wu, 1919)

Procedure

Preparation of protein free filtrate: 0.5 ml of serum will be taken in a tube, add 1.5 ml of water, 10% of 1ml sodium tungstate and 1 ml of 2/3N sulphuric acid will be added, mixed and centrifuged. The filtrate will be used as a protein free extract.

To the test tube add 1ml of protein free filtrate, 3ml of water 1ml of 1N NaOH and 1ml 1% picric acid will be added. The reaction mixture will be incubated for 15 minutes at room temperature and the absorbance will be measured at 520nm. Test tube without protein free filtrate will serve as blank. Creatinine is used as working standard.

II. **Estimation of glycosylated haemoglobin** (Nayak & Pattabhiraman,1981)

Procedure

The saline washed erythrocytes (0.5 mL) will be lysed with 5.5 mL of water, mixed and incubated at 37 °C for 15 minutes. The contents will be centrifuged and the supernatant will be discarded, then 0.5 mL of saline will be added, mixed and processed for estimation. To 0.02 mL of aliquot, 4 mL of oxalate hydrochloric solution will be added and mixed. The contents will be heated at 100°C for 4 hours, cooled and precipitated with 2 mL of 40% TCA. The mixture will be centrifuged and to 0.5 mL of supernatant, 0.05 mL of 80% phenol and 3.0 mL of concentrated H_2SO_4 will be added. The colour developed will be read at 480 nm after 30 minutes.

III. **Determination of Glucose-6-phosphatase activity** (Koida & Oda, 1959)

Procedure

Incubation mixture will be containing 0.7 mL of citrate buffer (pH 7.4), 0.3 mL of substrate, and 0.3 mL of tissue homogenate. The reaction mixture will be incubated at 37 °C for 1 h. Addition of 1 mL of 10% TCA to the reaction tubes terminated the reaction of the enzyme. The suspension will be centrifuged and the phosphorus content of the supernatant of tissue homogenate will be estimated by the method of Fiske and Subbarow (1925).The supernatant will be adjusted to known volume. To this1 mL of Ammonium molybdate (2.5%) will be added followed by 0.4 mL of ANSA (1-amino-2-napthol sulfonic acid). After 20 min the absorbance will be read at 680 nm.

IV. Metabolic indicators

1. **Estimation of liver glycogen** (Morales *et al*., 1973)

Procedure

A known amount of the tissue will be subjected to alkali digestion in a boiling water bath for 20 minutes after addition of 5 mL of 30% potassium hydroxide. The tubes will be cooled and 3.0 mL of absolute ethanol and a drop of Ammonium acetate will be added. The tubes will be then placed in a freezer overnight to precipitate the glycogen. The precipitated glycogen will be collected after centrifugation at 3000× *g* for 10 minutes. The precipitate will be washed thrice with alcohol and dissolved in 3 mL of water. Aliquots will be taken and 4 mL of anthrone reagent will be added to the tubes kept in an ice bath, mixed and heated in a boiling water bath for 20 minutes. The green colour developed will be read at 640 nm. Working standard glucose and a blank will be treated similarly.

2. **Estimation of cholesterol** (Zlatkies *et al*., 1953)

Procedure

Estimation of Cholesterol will be carried out by the method of Zlatkis *et al*„ (1953) *9.9* ml of ferric chloride -acetic acid reagent (0.05%) will be added to 0.1 ml of serum of deprotenization. The contents will be centrifuged at 3000rpm for 15 min. 5 ml of the supernatant will be taken and to this added 3 ml of conc. Sulphuric acid. Further kept for 20 min at room temperature. The pink colour will be formed and it is read at 540 nm. Cholesterol in aldehyde free acetic acid is used as standard.

V. Histopathalogical studies (Deb *et al.,* 2013; Bitencourt *et al* ., 2015)

Tissue specimens (pancreas, liver and kidney) from normal rats, diabetic rats, diabetic treated with plant extracts for a period of 21 days, will be collected after sacrificing the rats with cervical dislocation. The specimens will be fixed in neutralized formalin, dehydrated with ethanol and embedded in paraffin-wax (56°C). Serial sections (5μm) will be taken and stained with hematoxylin and eosine. To study basament membrane thickening sections will be stained by periodic acid Schiff reaction for light microscopy.

4. REFERENCES

1. Adefegha, S. A., & Oboh, G. (2012). In vitro inhibition activity of polyphenol-rich extracts from *Syzygium aromaticum* (L.) Merr. & Perry (Clove) buds against carbohydrate hydrolyzing enzymes linked to type 2 diabetes and Fe_2^+-induced lipid peroxidation in rat pancreas. *Asian Pacific journal of tropical biomedicine*, *2*(10), 774-781.
2. Agarwal, P., & Gupta, R. (2016). Alpha-amylase inhibition can treat diabetes mellitus. *Research and Reviews Journal of Medical and Health Sciences*, *5*(4), 1-8.
3. Ajiboye, B. O., Ojo, O. A., Akuboh, O. S., Abiola, O. M., Idowu, O., & Amuzat, A. O. (2018). Anti-Hyperglycemic and Anti-Inflammatory Activities of Polyphenolic-Rich Extract of *Syzygium cumini* Linn Leaves in Alloxan-Induced Diabetic Rats. *Journal of evidence-based integrative medicine*, *23*, 2515690X18770630.
4. Al-Shamaony, L., Al-Khazraji, S. M., & Twaij, H. A. (1994). Hypoglycaemic effect of *Artemisia herba alba*. II. Effect of a valuable extract on some blood parameters in diabetic animals. *Journal of ethnopharmacology*, *43*(3), 167-171.
5. Anjaneyulu, M., & Chopra, K. (2004). Nordihydroguairetic acid, a lignin, prevents oxidative stress and the development of diabetic nephropathy in rats. *Pharmacology*, *72*(1), 42-50.
6. Atale, N., Jaiswal, A., Chhabra, A., Malhotra, U., Kohli, S., Mohanty, S., & Rani, V. (2011). Phytochemical and antioxidant screening of *Syzygium cumini* seed extracts: a comparative study. *J Pharm Res*, *4*(12), 4530-4532.
7. Atawodi, S. E. (2005). Antioxidant potential of African medicinal plants. *African Journal of Biotechnology*, *4*(2), 128-133.
8. Attanayake, A. P., Jayatilaka, K. A., Pathirana, C., & Mudduwa, L. K. (2013). Study of antihyperglycaemic activity of medicinal plant extracts in alloxan induced diabetic rats. *Ancient Science of life*, *32*(4), 193.
9. Ayyanar, M., & Subash-Babu, P. (2012). *Syzygium cumini* (L.) Skeels: A review of its phytochemical constituents and traditional uses. *Asian Pacific journal of tropical biomedicine*, *2*(3), 240-246.
10. Babu, P. S., Prabuseenivasan, S., & Ignacimuthu, S. (2007). Cinnamaldehyde—a potential antidiabetic agent. *Phytomedicine*, *14*(1), 15-22.
11. Bairy, K. L., Sharma, A., & Shalini, A. (2005). Evaluation of the hypoglycemic, hypolipidaemic and hepatic glycogen raising effects of *Syzygium malaccense* upon streptozotocin induced diabetic rats. *Journal of Natural remedies*, *5*(1), 46-51.
12. Bajaj, S., & Khan, A. (2012). Antioxidants and diabetes. *Indian journal of endocrinology and metabolism*, *16*(Suppl 2), S267.
13. Bajpai, M., Pande, A., Tewari, S. K., & Prakash, D. (2005). Phenolic contents and antioxidant activity of some food and medicinal plants. *International Journal of Food Sciences and Nutrition*, *56*(4), 287-291.
14. Baliga, M. S., Fernandes, S., Thilakchand, K. R., D'souza, P., & Rao, S. (2013). Scientific validation of the antidiabetic effects of *Syzygium jambolanum* DC (black plum), a

traditional medicinal plant of India. *The Journal of Alternative and Complementary Medicine*, *19*(3), 191-197.

15. Banerjee, J., & Narendhirakannan, R. T. (2011). Biosynthesis of silver nanoparticles from *Syzygium cumini* (L.) seed extract and evaluation of their in vitro antioxidant activities. *Dig J Nanomater Biostruct*, *6*(3), 961-968.
16. Banerjee, S., Panda, C. K., & Das, S. (2006). Clove (*Syzygium aromaticum* L.), a potential chemopreventive agent for lung cancer. *Carcinogenesis*, *27*(8), 1645-1654.
17. Bansode, T. S., Gupta, A., & Salalkar, B. K. (2016). In silico and in vitro assessment on antidiabetic efficacy of secondary metabolites from *Syzygium cumini* (L.) Skeels. *Plant Science Today*, *3*(4), 360-367.
18. Bitencourt, P. E. R., De Bona, K. S., Cargnelutti, L. O., Bonfanti, G., Pigatto, A., Boligon, A.,& Moretto, M. B. (2015). *Syzygium cumini* seed extract ameliorates adenosine deaminase activity and biochemical parameters but does not alter insulin sensitivity and pancreas architecture in a short-term model of diabetes. *Journal of Complementary and Integrative Medicine*, *12*(3), 187-193.
19. Brahmachari, H. D., & Augusti, K. T. (1962). Orally effective hypoglycaemic agents from plants. *Journal of Pharmacy and Pharmacology*, *14*(1), 254-255.
20. Ceriello, Antonio. "New insights on oxidative stress and diabetic complications may lead to a "causal" antioxidant therapy." *Diabetes care* 26, no. 5 (2003): 1589-1596.
21. Chandran, R., Parimelazhagan, T., & George, B. P. (2017). Antihyperglycemic activity of the bark methanolic extract of *Syzygium mundagam* in diabetic rats. *Alexandria Journal of Medicine*, *53*(4), 317-324.
22. Chandran, R., Parimelazhagan, T., Shanmugam, S., & Thankarajan, S. (2016). Chandran, R., Parimelazhagan, T., Shanmugam, S., & Thankarajan, S. (2016). Antidiabetic activity of *Syzygium calophyllifolium* in Streptozotocin-Nicotinamide induced Type-2 diabetic rats. *Biomedicine & Pharmacotherapy*, *82*, 547-554.
23. Antidiabetic activity of Syzygium calophyllifolium in Streptozotocin-Nicotinamide induced Type-2 diabetic rats. *Biomedicine & Pharmacotherapy*, *82*, 547-554.
24. Chang, C. W., Wu, T. S., Hsieh, Y. S., Kuo, S. C., & Chao, P. D. L. (1999). Terpenoids of *Syzygium formosanum*. *Journal of natural products*, *62*(2), 327-328.
25. Chattopadhyay RR, Medd CS, Das S, Basu TK, Podder G (1993). Hypoglycaemic and anti-hyperglycaemic effect of *Gymnema sylvestre* leaf extract in rats. Fitoterapia 64:450–454
26. Deb, L., Bhattacharjee, C., Shetty, S. R., & Dutta, A. (2013). *Syzygium cuminii* (linn) skeels by reverse pharmacological approacheS. *Bulletin of Pharmaceutical Research*, *3*(3), 135-45.
27. Deliwe, M., & Amabeoku, G. J. (2013). Evaluation of the antidiarrhoeal and antidiabetic activities of the leaf aqueous extract of *Syzygium cordatum* hoscht. ex C. Krauss (Mytraceae) in rodents.
28. Djoukeng, J. D., Abou-Mansour, E., Tabacchi, R., Tapondjou, A. L., Bouda, H., & Lontsi, D. (2005). Antibacterial triterpenes from *Syzygium guineense* (Myrtaceae). *Journal of Ethnopharmacology*, *101*(1-3), 283-286.

29. Ezenyi, I. C., Mbamalu, O. N., Balogun, L., Omorogbe, L., Ameh, F. S., & Salawu, O. A. (2016). Antidiabetic potentials of *Syzygium guineense* methanol leaf extract. *J. Phytopharmacol*, *5*, 150-156.
30. Fabricant, D. S., & Farnsworth, N. R. (2001). The value of plants used in traditional medicine for drug discovery. *Environmental health perspectives*, *109*(Suppl 1), 69.
31. Fenercioglu, A. K., Saler, T., Genc, E., Sabuncu, H., & Altuntas, Y. (2010). The effects of polyphenol-containing antioxidants on oxidative stress and lipid peroxidation in Type 2 diabetes mellitus without complications. *Journal of endocrinological investigation*, *33*(2), 118-124.
32. Fiske, C. H., & Subbarow, Y. (1925). The colorimetric determination of phosphorus. *J. biol. Chem*, *66*(2), 375-400.
33. Fluckiger, R., & Winterhalter, K. H. (1976). In vitro synthesis of haemoglobin A1c. *Febs Lett*, *71*(2), 356-360
34. Folin, O., & Wu, H. (1919). A system of blood analysis. *J. biol. Chem*, *38*(81), 116.
35. Fukai, T., & Ushio-Fukai, M. (2011). Superoxide dismutases: role in redox signaling, vascular function, and diseases. *Antioxidants & redox signaling*, *15*(6), 1583-1606.
36. Gavillán-Suárez, J., Aguilar-Perez, A., Rivera-Ortiz, N., Rodríguez-Tirado, K., Figueroa-Cuilan, W., Morales-Santiago, L., & Martínez-Montemayor, M. M. (2015). Chemical profile and in vivo hypoglycemic effects of *Syzygium jambos*, Costus speciosus and Tapeinochilos ananassae plant extracts used as diabetes adjuvants in Puerto Rico. *BMC complementary and alternative medicine*, *15*(1), 244.
37. George F, Cahill JR. *Current concepts on diabetes in Joslins diabetes mellitus*, Edited by Alexander christlieb, J. Stuart soeldner. K.M. Varghese Company, Bombay; 1985:1.
38. Ghosh, S., & Suryawanshi, S. A. (2001). Effect of *Vinca rosea* extracts in treatment of alloxan diabetes in male albino rats.
39. Ghoshal, K. P., & Saoji, A. A. (2013). Phytochemical screening of the pollen of some selected plants with antidiabetic properties. *Aust. J. Basic Appl. Sci*, *7*(7), 105-109.
40. Giugliano, D., Ceriello, A., & Paolisso, G. (1996). Oxidative stress and diabetic vascular complications. *Diabetes care*, *19*(3), 257-267.
41. Halliwell, B., & Gutteridge, J. M. (1990). [1] Role of free radicals and catalytic metal ions in human disease: an overview. In *Methods in enzymology* (Vol. 186, pp. 1-85). Academic Press.
42. Harborne, J. B. (1973). "Phytochemical Methods"; Chapman and Hall: New York, Jayaprasad B, Sharavanan PS,Sivaraj R. Antidiabetic effect of *Chloroxylon swietenia* bark extracts on streptozotocin induced diabetic rats. *Journal of basic and applied sciences.* 5 :61–69
43. Helmstädter, A. (2008). *Syzygium cumini* (L.) SKEELS (Myrtaceae) against diabetes–125 years of research. *Die Pharmazie-An International Journal of Pharmaceutical Sciences*, *63*(2), 91-101.
44. Herman, W. H., & Zimmet, P. (2012). Type 2 diabetes: an epidemic requiring global attention and urgent action. *Diabetes care*, *35*(5), 943-944.
45. Hermansen, K., & Mortensen, L. S. (2007). Bodyweight changes associated with antihyperglycaemic agents in type 2 diabetes mellitus. *Drug Safety*, *30*(12), 1127-1142.

46. Holman, R. R., & Turner, R. C. (1991). Oral agents and insulin in the treatment of NIDDM. *Textbook of Diabetes. Blackwell, Oxford*, 407-469.
47. Horii, S., Fukase, H., Matsuo, T., Kameda, Y., Asano, N., & Matsui, K. (1986). Synthesis and. alpha.-D-glucosidase inhibitory activity of N-substituted valiolamine derivatives as potential oral antidiabetic agents. *Journal of medicinal chemistry*, *29*(6), 1038-1046.
48. Huang, D. W., Chang, W. C., Wu, J. S. B., Shih, R. W., & Shen, S. C. (2016). Vescalagin from Pink wax apple [*Syzygium samarangense* (Blume) Merrill and Perry] alleviates hepatic insulin resistance and ameliorates glycemic metabolism abnormality in rats fed a high-fructose diet. *Journal of agricultural and food chemistry*, *64*(5), 1122-1129.
49. Jagetia, G. C. (2018). A review on the role of jamun, *Syzygium cumini* skeels in the treatment of diabetes. *Int J Complement Alt Med*, *11*(2), 91-95.
50. Jakus, V. (2000). The role of free radicals, oxidative stress and antioxidant systems in diabetic vascular disease. *Bratislavske lekarske listy*, *101*(10), 541-551.
51. Jeyasankar, A., Raja, N., & Ignacimuthu, S. (2011). Insecticidal compound isolated from *Syzygium lineare* Wall.(Myrtaceae) against Spodoptera litura (Lepidoptera: Noctuidae). *Saudi journal of biological sciences*, *18*(4), 329-332.
52. Jirovetz L, Buchbauer G, Puschmann Ch, Fleischhacker W,Shafi PM, Rosamma MK.J. *Essent. Oil Bearing Plants*, 1999; *2*(2): 68 – 77
53. Joshi,R. K. & Shenoy, H.S & Marati, Ramakrishna. (2017). Chemical Composition of the Essential oil of *Syzygium kanarense*: An Endemic and Rediscovered Species from the Western Ghats, India. *Natural product communications*. 12. 1943-1944.
54. Kasetti, R. B., Rajasekhar, M. D., Kondeti, V. K., Fatima, S. S., Kumar, E. G. T., Swapna, S., & Rao, C. A. (2010). Antihyperglycemic and antihyperlipidemic activities of methanol: water (4: 1) fraction isolated from aqueous extract of *Syzygium alternifolium* seeds in streptozotocin induced diabetic rats. *Food and Chemical Toxicology*, *48*(4), 1078-1084.
55. Khathi, A., Serumula, M. R., Myburg, R. B., Van Heerden, F. R., & Musabayane, C. T. (2013). Effects of *Syzygium aromaticum*-derived triterpenes on postprandial blood glucose in streptozotocin-induced diabetic rats following carbohydrate challenge. *PLoS One*, *8*(11), e81632.
56. Kirtikar, K.R, Basu, B.D, *Indian medicinal plants*, International book distributors, Dehradun, 2006, 993-994
57. Koida H, Oda T., (1959). Pathological occurrence of glucose-6- phosphatase in tissue homogenate in liver diseases. *Clin Chim Acta*; 4:554–61
58. Konczak, I., Zabaras, D., Dunstan, M., & Aguas, P. (2010). Antioxidant capacity and hydrophilic phytochemicals in commercially grown native Australian fruits. *Food Chemistry*, *123*(4), 1048-1054.
59. Krishnasamy, G., & Muthusamy, K. (2015). *In vitro* evaluation of antioxidant and antidiabetic activities of *Syzygium densiflorum* fruits. *Asian Pacific Journal of Tropical Disease*, *5*(11), 912-917.
60. Krishnasamy, G., Muthusamy, K., Chellappan, D. R., & Subbiah, N. (2016). Antidiabetic, antihyperlipidaemic, and antioxidant activity of *Syzygium densiflorum* fruits in streptozotocin and nicotinamide-induced diabetic rats. *Pharmaceutical biology*, *54*(9), 1716-1726.

61. Kumar, A., Ilavarasan, R., Deecaraman, M., Aravindan, P., Padmanabhan, N., & Krishan, M. R. V. (2013). Anti-diabetic activity of *Syzygium cumini* and its isolated compound against streptozotocin-induced diabetic rats. *Journal of Medicinal Plants Research*, *2*(9), 246-249.
62. Kumar, A., Ilavarasan, R., Jayachandran, T., Decaraman, M., Aravindhan, P., Padmanabhan, N., & Krishnan, M. R. V. (2009). Phytochemicals investigation on a tropical plant, *Syzygium cumini* from Kattuppalayam, Erode district, Tamil Nadu, South India. *Pakistan Journal of Nutrition*, *8*(1), 83-85.
63. Kusuma, I. W., Kuspradini, H., Arung, E. T., Aryani, F., Min, Y. H., Kim, J. S., & Kim, Y. U. (2011). Biological activity and phytochemical analysis of three Indonesian medicinal plants, *Murraya koenigii*, *Syzygium polyanthum* and *Zingiber purpurea*. *Journal of Acupuncture and Meridian Studies*, *4*(1), 75-79.
64. Lorenzati, B., Zucco, C., Miglietta, S., Lamberti, F., & Bruno, G. (2010). Oral hypoglycemic drugs: pathophysiological basis of their mechanism of actionoral hypoglycemic drugs: pathophysiological basis of their mechanism of action. *Pharmaceuticals*, *3*(9), 3005-3020.
65. Luvuno, M., Mbongwa, H. P., & Khathi, A. (2016). The effects of *syzygium aromaticum*-derived triterpenes on gastrointestinal ghrelin expression in streptozotocin-induced diabetic rats. *African Journal of Traditional, Complementary and Alternative Medicines*, *13*(4), 8-14.
66. Mabberly D J (2008), Plant Book III edition. Cambridge university press.
67. MacDonald-Wicks, L. K., Wood, L. G., & Garg, M. L. (2006). Methodology for the determination of biological antioxidant capacity *in vitro*: a review. *Journal of the Science of Food and Agriculture*, *86*(13), 2046-2056
68. Manaharan, T., Appleton, D., Cheng, H. M., & Palanisamy, U. D. (2012). Flavonoids isolated from *Syzygium aqueum* leaf extract as potential antihyperglycaemic agents. *Food Chemistry*, *132*(4), 1802-1807.
69. Manaharan, T., Ming, C. H., & Palanisamy, U. D. (2013). Syzygium aqueum leaf extract and its bioactive compounds enhances pre-adipocyte differentiation and 2-NBDG uptake in 3T3-L1 cells. *Food chemistry*, *136*(2), 354-363.
70. Matsuoka T, Kajimoto Y, Watada H, Kaneto H, Kishimoto M, Umayahara Y, et al. Glycation-dependent, reactive oxygen speciesmediated suppression of the insulin gene promoter activity in HIT cells. *J Clin Invest* 1997; 99:144–50.
71. McCrimmon, R. J., & Sherwin, R. S. (2010). Hypoglycemia in type 1 diabetes. *Diabetes*, *59*(10), 2333-2339.
72. Mensor LL Menezes FS, Leitão GG, Reis AS, dos Santos TC, Coube CS, Leitão SG.(2001) Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytother Res.* 15(2):127-30.
73. Mittal, M., Gupta, N., Parashar, P., Mehra, V., & Khatri, M. (2014). Phytochemical evaluation and pharmacological activity of *Syzygium aromaticum*: a comprehensive review. *International Journal of Pharmacy and Pharmaceutical Sciences*, *6*(8), 67-72.
74. Mittal, M., Gupta, N., Parashar, P., Mehra, V., & Khatri, M. (2014). Phytochemical evaluation and pharmacological activity of *Syzygium aromaticum*: a comprehensive review. *International Journal of Pharmacy and Pharmaceutical Sciences*, *6*(8), 67-72.

75. Moon, J. K., & Shibamoto, T. (2009). Antioxidant assays for plant and food components. *Journal of agricultural and food chemistry*, *57*(5), 1655-1666.
76. Musabayane, C. T., Mahlalela, N., Shode, F. O., & Ojewole, J. A. O. (2005). Effects of *Syzygium cordatum* (Hochst.)[Myrtaceae] leaf extract on plasma glucose and hepatic glycogen in streptozotocin-induced diabetic rats. *Journal of Ethnopharmacology*, *97*(3), 485-490.
77. Muthumperumal, C., Stalin, N., Das, A., & Swamy, P. S. (2016). Chemical profiling of leaf essential oil, Antioxidant potential and Antibacterial activity of *Syzygium lanceolatum* (Lam.) Wt. & Arn.(Myrtaceae). *Free Radicals & Antioxidants*, *6*(1).
78. Nagulendran, K. R., Velavan, S., Mahesh, R., & Begum, V. H. (2007). In vitro antioxidant activity and total polyphenolic content of *Cyperus rotundus* rhizomes. *Journal of Chemistry*, *4*(3), 440-449.
79. Nakagawa-Goto, K., Yamada, K., Taniguchi, M., Tokuda, H., & Lee, K. H. (2009). Cancer preventive agents 9. Betulinic acid derivatives as potent cancer chemopreventive agents. *Bioorganic & medicinal chemistry letters*, *19*(13), 3378-3381.
80. Nayak, SS, Pattabiraman, TN. (1981). A new colorimetric method for the estimation of glycosylated hemoglobin. *Clin Chim Acta.*; 109:267–274.
81. Naziroğlu, M., & Butterworth, P. J. (2005). Protective effects of moderate exercise with dietary vitamin C and E on blood antioxidative defense mechanism in rats with streptozotocin-induced diabetes. *Canadian journal of applied physiology*, *30*(2), 172-185.
82. Nelson DC, Cox MM. (2010). *Lehninger Principles of Biochemistry*. 4th Ed. New York: W.H. Freeman and Co.,
83. Oboh, G., Raddatz, H., & Henle, T. (2008). Antioxidant properties of polar and non-polar extracts of some tropical green leafy vegetables. *Journal of the Science of Food and Agriculture*, *88*(14), 2486-2492.
84. Osman, H., Rahim, A. A., Isa, N. M., & Bakhir, N. M. (2009). Antioxidant activity and phenolic content of *Paederia foetida* and *Syzygium aqueum*. *Molecules*, *14*(3), 970-978.
85. Pari L, Latha M. (2005). Antidiabetic effect of *Scoparia dulcis*: effect on lipid peroxidation in streptozotocin diabetes, *Gen Physiol Biophys*, *24*,13-26.
86. Patel, B. D., & Kori, M. L. (2018). Anti-diabetic activity of *Albizia amara boivin* leaf on streptozotocin induced diabetes in rats. *International Journal of Pharmaceutical and Biological Science Archive*, *6*(03).
87. Penckofer, S., Schwertz, D., & Florczak, K. (2002). Oxidative stress and cardiovascular disease in type 2 diabetes: the role of antioxidants and pro-oxidants. *Journal of Cardiovascular Nursing*, *16*(2), 68-85.
88. Ponnusamy, S., Ravindran, R., Zinjarde, S., Bhargava, S., & Ravi Kumar, A. (2011). Evaluation of traditional Indian antidiabetic medicinal plants for human pancreatic amylase inhibitory effect in vitro. *Evidence-Based Complementary and Alternative Medicine*, *2011*.
89. Radha, R., Latha, R., & Swaminathan, M. S. (2002). Chemical composition and bioactivity of essential oil from *Syzygium travancoricum* Gamble. *Flavour and fragrance journal*, *17*(5), 352-354.

90. Raffaelli, F., Borroni, F., Alidori, A., Tirabassi, G., Faloia, E., Rabini, R. A., & Vignini, A. (2015). Effects of in vitro supplementation with *Syzygium cumini* (L.) on platelets from subjects affected by diabetes mellitus. *Platelets*, *26*(8), 720-725.
91. Rao, B. K., Kesavulu, M. M., & Apparao, C. (2001). Antihyperglycemic activity of *Momordica cymbalaria* in alloxan diabetic rats. *Journal of Ethnopharmacology*, *78*(1), 67-71.
92. Rao.PS, Krishnamohan G. (2017). *In vitro* alpha-amylase inhibition and *in vivo* antioxidant potential of *Momordica dioica* seeds in streptozotocin-induced oxidative stress in diabetic rats. *Saudi Journal of Biological Sciences*.*24*(6), 1262-126
93. Rastogi, R. P., & Mehrotra, B. N. (1995). Compendium of Indian medicinal plants: *Lucknow, India: Central Drug Research Institute 930p. ISBN 8185042136 En chemical_analysis, Asia_Tropical, medicines, Indian_Subcontinent, morphology, laboratory_tests, pharmacology, vernacular_names, distribution, India , 4*, 1985-1989.
94. Ravi, K., Rajasekaran, S., & Subramanian, S. (2005). Antihyperlipidemic effect of *Eugenia jambolana* seed kernel on streptozotocin-induced diabetes in rats. *Food and Chemical Toxicology*, *43*(9), 1433-1439.
95. Ravi, K., Sivagnanam, K., & Subramanian, S. (2004). Anti-diabetic activity of *Eugenia jambolana* seed kernels on streptozotocin-induced diabetic rats. *Journal of medicinal food*, *7*(2), 187-191
96. Resurreccion-Magno, M. H. C., Villaseñor, I. M., Harada, N., & Monde, K. (2005). Antihyperglycaemic flavonoids from *Syzygium samarangense* (Blume) merr. And perry. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *19*(3), 246-251.
97. Reynertson, K. A., Yang, H., Jiang, B., Basile, M. J., & Kennelly, E. J. (2008). Quantitative analysis of antiradical phenolic constituents from fourteen edible Myrtaceae fruits. *Food Chemistry*, *109*(4), 883-890.
98. Ruch, R. J., Cheng, S. J., & Klaunig, J. E. (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*, *10*(6), 1003-1008.
99. Sabu, M. C., & Kuttan, R. (2002). Anti-diabetic activity of medicinal plants and its relationship with their antioxidant property. *Journal of ethnopharmacology*, *81*(2), 155-160.
100. Sagrawat, H. (2006). Pharmacological potential of *Eugenia jambolana*: A review. *Pharmacogn Mag*, *2*(6), 96-105.
101. Sahana, D. A., Shivaprakash, G., Baliga, R., MR, A. P., Ganesh, J., & Pai, M. R. S. M. (2010). Effect of *Eugenia jambolana* on plasma glucose, insulin sensitivity and HDL-C levels: preliminary results of a randomized clinical trial. *Journal of Pharmacy Research*, *3*(6), 1268-1270.
102. Sajeeth C., Manna P.K and Manavalan R, (2011). Antioxidant Activity of Polyherbal Formulation on Streptozotocin Induced Diabetes in Experimental Animals. *Pelagia Research Library Der Pharmacia Sinica*.2 (2): 220-22
103. Samadder, A., Chakraborty, D., De, A., Bhattacharyya, S. S., Bhadra, K., & Khuda-Bukhsh, A. R. (2011). Possible signaling cascades involved in attenuation of alloxan-induced oxidative stress and hyperglycemia in mice by ethanolic extract of *Syzygium*

jambolanum: drug-DNA interaction with calf thymus DNA as target. *European Journal of Pharmaceutical Sciences*, *44*(3), 207-217.

104. Sandikapura, M. J., Nyamathulla, S., & Noordin, M. I. (2018). Comparative antioxidant and antidiabetic effects of *Syzygium polyanthum* leaf and *Momordica charantia* fruit extracts. *Pakistan journal of pharmaceutical sciences*, *31*.
105. Savitha, R. C., Padmavathy, S., & Sundhararajan, A. (2011). *In vitro* antioxidant activities on leaf extracts of *Syzygium malaccense* (L.) merr and perry. *Ancient science of life*, *30*(4), 110.
106. Sawant, L., Singh, V. K., Dethe, S., Bhaskar, A., Balachandran, J., Mundkinajeddu, D., & Agarwal, A. (2015). Aldose reductase and protein tyrosine phosphatase 1B inhibitory active compounds from *Syzygium cumini* seeds. *Pharmaceutical biology*, *53*(8), 1176-1182.
107. Schossler, D. R. C., Mazzanti, C. M., Luz, S. C. A. D., Filappi, A., Prestes, D., Silveira, A. F. D., & Cecim, M. (2004). *Syzygium cumini* and the regeneration of insulin positive cells from the pancreatic duct. *Brazilian Journal of Veterinary Research and Animal Science*, *41*(4), 236-239.
108. Senggunprai, L., Kukongviriyapan, V., Prawan, A., & Kukongviriyapan, U. (2010). Consumption of *Syzygium gratum* promotes the antioxidant defense system in mice. *Plant foods for human nutrition*, *65*(4), 403-409.
109. Shafi, P. M., Rosamma, M. K., Jamil, K., & Reddy, P. S. (2002). Antibacterial activity of *Syzygium cumini* and Syzygium travancoricum leaf essential oils. *fitoterapia*, *73*(5), 414-416.
110. Shahreen, S., Banik, J., Hafiz, A., Rahman, S., Zaman, A. T., Shoyeb, A., & Rahmatullah, M. (2012). Antihyperglycemic activities of leaves of three edible fruit plants (*Averrhoa carambola, Ficus hispida* and *Syzygium samarangense*) of Bangladesh. *African Journal of Traditional, Complementary and Alternative Medicines*, *9*(2), 287-291.
111. Sharafeldin, K., & Rizvi, M. R. (2015). Effect of traditional plant medicines (*Cinnamomum zeylanicum* and *Syzygium cumini*) on oxidative stress and insulin resistance in streptozotocin-induced diabetic rats. *The Journal of Basic & Applied Zoology*, *72*, 126-134.
112. Shareef, S. M., Santhosh Kumar, E. S., & Shaju, T. (2012). A new species of *Syzygium* (Myrtaceae) from the southern Western Ghats of Kerala, India. *Phytotaxa*, *71*(1), 28-33.
113. Sharma, B., Balomajumder, C., & Roy, P. (2008). Hypoglycemic and hypolipidemic effects of flavonoid rich extract from *Eugenia jambolana* seeds on streptozotocin induced diabetic rats. *Food and chemical toxicology*, *46*(7), 2376-2383.
114. Shaw, J. E., Sicree, R. A., & Zimmet, P. Z. (2010). Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes research and clinical practice*, *87*(1), 4-14.
115. Shen, S. C., & Chang, W. C. (2013). Hypotriglyceridemic and hypoglycemic effects of vescalagin from Pink wax apple [*Syzygium samarangense* (Blume) Merrill and Perry cv. Pink] in high-fructose diet-induced diabetic rats. *Food chemistry*, *136*(2), 858-863.
116. Shilpa, K. J., & Krishnakumar, G. (2015). Nutritional, fermentation and pharmacological studies of *Syzygium caryophyllatum* (L.) Alston and *Syzygium zeylanicum* (L.) DC fruits. *Cogent Food & Agriculture*, *1*(1), 1018694.

117. Shinde, J., Taldone, T., Barletta, M., Kunaparaju, N., Hu, B., Kumar, S., & Zito, S. W. (2008). α-Glucosidase inhibitory activity of *Syzygium cumini* (Linn.) Skeels seed kernel in vitro and in Goto–Kakizaki (GK) rats. *Carbohydrate Research*, *343*(7), 1278-1281.
118. Sicree, R., Shaw, J., & Zimmet, P. (2006). Prevalence and projections. *Diabetes atlas*, *3*, 16-104.
119. Sies, H. (1985). Oxidative stress: introductory remarks. *Oxidative stress*, *501*, 1-8.
120. Singh, N., & Gupta, M. (2007). Effects of ethanolic extract of *Syzygium cumini* (Linn) seed powder on pancreatic islets of alloxan diabetic rats.
121. Srinivasan, K. (2005). Plant foods in the management of diabetes mellitus: spices as beneficial antidiabetic food adjuncts. *International journal of food sciences and nutrition*, *56*(6), 399-414.
122. Swaroopa, P., & Code, Q. R. (2017). Review on Antidiabetic Activity on Medicinal Plants. *IJPR*, *7*(12).
123. Tahir, H. U., Sarfraz, R. A., Ashraf, A., & Adil, S. (2016). Chemical Composition and Antidiabetic Activity of Essential Oils Obtained from Two Spices (*Syzygium aromaticum* and *Cuminum cyminum*). *International journal of food properties*, *19*(10), 2156-2164.
124. Trimurtulu, G., & Raghavendra, N. M. (2015). Isolation, in vitro antidiabetic, antioxidant activity and molecular docking studies of pentacyclic triterpenoids from *Syzygium alternifolium* (wt.) Walp bark. *Journal of Pharmacy and Biological Sciences*, *10*(6), 22-27.
125. Trinder P. (1969).Determination of blood glucose using an oxidase peroxidase system with a non-carcinogen chromogen. *J Clin Pathol*.22:158
126. Tripathi, A. K., & Kohli, S. (2014). Pharmacognostical standardization and antidiabetic activity of *Syzygium cumini* (Linn.) barks (Myrtaceae) on streptozotocin-induced diabetic rats. *Journal of Complementary and Integrative Medicine*, *11*(2), 71-81.
127. Tundis, R., Loizzo, M. R., & Menichini, F. (2010). Natural products as α-amylase and α-glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: an update. *Mini reviews in medicinal chemistry*, *10*(4), 315-331.
128. Vats, V., Yadav, S. P., & Grover, J. K. (2004). Ethanolic extract of *Ocimum sanctum* leaves partially attenuates streptozotocin-induced alterations in glycogen content and carbohydrate metabolism in rats. *Journal of ethnopharmacology*, *90*(1), 155-160.
129. Veeresham C. (2012). Natural products derived from plants as a source of drugs. *Journal of advanced pharmaceutical technology & research*, *3*(4), 200-1.
130. Vinodkumar, T. G. (2015). Leaf essential oil composition of six *Syzygium* Species from the Western Ghats, South India. *Records of natural products*, *9*(4), 592.
131. Vlietinck, A. J., & Berghe, D. A. V. (1991). Can ethnopharmacology contribute to the development of antiviral drugs?. *Journal of ethnopharmacology*, *32*(1-3), 141-153.
132. Warrier, P. K., Nambiar, V. P. K., & Ramankutty, C. (1994). *Indian Medicinal Plants: A Compendium of 500 species*. Orient Longman Publishers. *Kottakkal, India*, *2*.
133. WHO 2002. *World Health Organization's Traditional Medicine Strategy* 2002-2005. Geneva: WHO.
134. Widharna, R. M., Tamayanti, W. D., Hendriati, L., Hamid, I. S., & Widjajakusuma, E. C. (2015). Antidiabetic effect of the aqueous extract mixture of *Andrographis paniculata* and *Syzygium polyanthum* leaf. *European Journal of Medicinal Plants*, *6*(2), 82.

135. Widyawati, T., Yusoff, N. A., Asmawi, M. Z., & Ahmad, M. (2015). Antihyperglycemic effect of methanol extract of *Syzygium polyanthum* (Wight.) leaf in streptozotocin-induced diabetic rats. *Nutrients*, *7*(9), 7764-7780.
136. Wilson, P. G., O'Brien, M. M., Gadek, P. A., & Quinn, C. J. (2001). Myrtaceae revisited: a reassessment of infrafamilial groups. *American Journal of Botany*, *88*(11), 2013-2025.
137. Yokozawa, T., Kim, H. Y., & Cho, E. J. (2002). Erythritol attenuates the diabetic oxidative stress through modulating glucose metabolism and lipid peroxidation in streptozotocin-induced diabetic rats. *Journal of agricultural and food chemistry*, *50*(19), 5485-5489.
138. You, W. P., & Henneberg, M. (2016). Type 1 diabetes prevalence increasing globally and regionally: the role of natural selection and life expectancy at birth. *BMJ Open Diabetes Research and Care*, *4*(1), e000161.
139. Zlatikis, A. Zak, B and A.J Boyle (1953). A new method for the direct determination of serum cholesterol. *J. Of lab. Clin Med*. 41: 486-92